aProximate Primary proximal tubule cell models for drug development safety assessment and Nephrotoxicity studies

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We have developed a primary culture model of human proximal tubule cell monolayers.

In parallel we have developed a rat proximal tubule cell model.

**STRATEGY**

Screen NCEs through rat and human proximal tubule models

Identify compounds with renal liability in either species

Proceed into animal testing with compounds with no apparent liabilities in human kidney

Development of models supported by grant funding from

AstraZeneca

NC3Rs

Kidney Research UK
Human Kidney as a Source of Cells

Kidney – Transplant grade tissue less than 18 hrs ex-vivo.
Full histories 0-76 years old male and female.
1-2 whole kidneys per week.
Isolation of human and rat PTCs

Cortex excised
Cortical slices taken from rat or human kidneys and minced.

Collagenase digestion
Minced tissue incubated with collagenase for 2 hours at 37 °C.

Density Separation
PTCs separated from heterogeneous cell populations using Percoll gradients.

Cell culture
Isolated PTCs seeded on plastics and Transwell inserts and grown until confluent monolayer formation.
Cells Grown as Polarised Monolayers on Filter Supports

Screen: Drug secretion & Absorption; Drug toxicity, Drug-Drug interactions
aProximate™ PT cells Remain Extremely Well Differentiated

Key Transporter Expression

- mRNA levels ~ 30% of fresh tissue levels
- c.f. immortalised human kidney cell lines 1-5% and many at 0% expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Percentage of native kidney expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human PTC</td>
</tr>
<tr>
<td>MDR1</td>
<td>65.2 ± 7.1</td>
</tr>
<tr>
<td>BCRP</td>
<td>31.3 ± 5.5</td>
</tr>
<tr>
<td>MRP2</td>
<td>31.5 ± 33</td>
</tr>
<tr>
<td>MRP4</td>
<td>29.3 ± 4.8</td>
</tr>
<tr>
<td>OAT1</td>
<td>20.6 ± 4.6</td>
</tr>
<tr>
<td>OAT3</td>
<td>27.8 ± 6.7</td>
</tr>
<tr>
<td>OCT2</td>
<td>39.7 ± 4.3</td>
</tr>
<tr>
<td>OATP4C1</td>
<td>39.0 ± 2.7</td>
</tr>
<tr>
<td>SLC2A9</td>
<td>27.7 ± 4.8</td>
</tr>
<tr>
<td>URAT1</td>
<td>34.6 ± 9.2</td>
</tr>
<tr>
<td>MATE1</td>
<td>36.4 ± 4.2</td>
</tr>
<tr>
<td>MATE2K</td>
<td>30.1 ± 8.8</td>
</tr>
</tbody>
</table>
Measurement of TEER values in Proximal Tubule Models

<table>
<thead>
<tr>
<th>Tubule Cells</th>
<th>TEER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human proximal tubule cells</td>
<td>114.2 ± 4.7 Ω.cm²</td>
</tr>
<tr>
<td>Rat proximal tubule cells</td>
<td>98.6 ± 6.1 Ω.cm²</td>
</tr>
<tr>
<td>RPTEC (Evercyte)</td>
<td>154.4 ± 8.2 Ω.cm²</td>
</tr>
<tr>
<td>HPTEC (Evercyte)</td>
<td>no resistance</td>
</tr>
<tr>
<td>ciPTEC</td>
<td>no resistance</td>
</tr>
<tr>
<td>HK2</td>
<td>no resistance</td>
</tr>
</tbody>
</table>
What is the Variability between Individual Kidneys?

mean flux 24.37 ± 1.13 pmol/cm²/hr  n= 30 kidneys

Lower 95% CL of mean 22.10
Higher 95% CL of mean 26.65
Measurement of Transepithelial Fluxes

Transcellular Flux

- Apical to Basolateral: Secretion
  - $J_{a-b}$
- Basolateral to Apical: Absorption
  - $J_{b-a}$

Paracellular Flux

- Apical to Basolateral: Mannitol
  - $J_{a-b}$
- Basolateral to Apical: Mannitol
  - $J_{b-a}$
Examples of PTCs being Predictive of Renal Handling

Creatinine Organic Cations

Urate Organic Anions

Phosphate

Examples of PTCs being Predictive of Renal Handling
PTCs retain Megalin/Cubilin expression

Megalin/Cubilin are key for receptor-mediated endocytosis
<table>
<thead>
<tr>
<th>Endogenous Substrates</th>
<th>Xenobiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>α2-microalbumin</td>
<td>Aprotinin</td>
</tr>
<tr>
<td>β-microalbumin</td>
<td>Polymyxins</td>
</tr>
<tr>
<td>Apolipoproteins</td>
<td>Rifamycin</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>Trichosanthin</td>
</tr>
<tr>
<td>Intrinsic factor-vitamin B12</td>
<td>Oligonucleotides</td>
</tr>
<tr>
<td>Insulin</td>
<td>siRNA</td>
</tr>
<tr>
<td>Lysozyme</td>
<td></td>
</tr>
<tr>
<td>Prolactin</td>
<td></td>
</tr>
<tr>
<td>RAP</td>
<td></td>
</tr>
<tr>
<td>Retinol binding protein</td>
<td></td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td></td>
</tr>
<tr>
<td>Transcobalamin-vitamin B12</td>
<td></td>
</tr>
<tr>
<td>Transferrin</td>
<td></td>
</tr>
<tr>
<td>Transthyretin</td>
<td></td>
</tr>
<tr>
<td>Vitamin D binding protein</td>
<td></td>
</tr>
</tbody>
</table>

Substrates for Megalin and Cubulin in Kidney
Apical Uptake of Small Molecular Weight Proteins in Human PTC monolayers by Megalin/Cubulin

Substrates for the megalin/cublin uptake pathway
## Renal Biomarker Strategy

<table>
<thead>
<tr>
<th>Glomerulus</th>
<th>Proximal Tubule</th>
<th>Distal Tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>KIM 1, Clusterin, TF3*</td>
<td>TF3*, αGST, FABP</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>NGAL, NAG, IL 18, α GST**</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2M</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FDA/EMEA Guideline Biomarkers of Nephrotoxicity in Preclinical Studies</strong></td>
<td><strong>FDA/EMEA Guideline Biomarkers of Nephrotoxicity in Preclinical Studies</strong></td>
<td><strong>Emerging New Biomarkers of Nephrotoxicity in Preclinical Studies</strong></td>
</tr>
</tbody>
</table>

* TF3 is not well validated in vivo

α GST** is not very stable in urine
Renal Biomarker Multiplex  Cisplatin

**Clusterin**

**KIM-1**

**NGAL**

**TEER values**

**MATE1**

**MATE2K**

**OCT2**

**OCT3**
Concentration Dependence of Biomarkers of Proximal Tubule Toxicity

Amount of renal injury markers after 48 hr cisplatin treatment in human PTCs

- **Medium**
  - Clusterin
  - KIM-1
  - NGAL

- **Cell lysate**
  - Clusterin
  - KIM-1
  - NGAL
Renal Biomarker Multiplex: Methotrexate

Clusterin, KIM-1, NGAL

TEER VALUES

OAT1, OAT3, OAT2
Renal Biomarker Multiplex: Gentamycin

**Clusterin**

- Control
- 200 μg/ml Gentamicin

**KIM-1**

- Control
- 200 μg/ml Gentamicin

**NGAL**

- Control
- 200 μg/ml Gentamicin

**TEER Values**

- No treatment
- 200 μg/ml Gentamicin

**Number of days with treatment**

0 1 2 3 4 5

**TEER (Ω·cm²)**

0 20 40 60 80 100 120 140

**Clusterin**

**Megalgin**
Renal Biomarker Strategy: rPTC Gentamycin

As in human:
Changes in biomarker secretion  
before changes in TEER values  
or MTS measurement of cell viability
Renal Biomarker Strategy: rat PTC Polymyxin B

48hrs exposure to Polymyxin B

In rat proximal tubule monolayers:
Concentration dependent increase in KIM-1 in response to polymyxin B exposure
Concentration dependent loss of monolayer integrity in response to polymyxin B exposure
Renal Biomarker Strategy: human PTC Polymyxin B

48hrs exposure to Polymyxin B

In human proximal tubule monolayers:
Concentration dependent increase in KIM-1 in response to polymyxin B exposure
Concentration dependent loss of monolayer integrity in response to polymyxin B exposure
Renal Biomarker Strategy: human PTC Polymyxin B

In human proximal tubule monolayers:
Time and concentration dependent increase in KIM-1 in response to polymyxin exposure
Apical Uptake of Small Molecular Weight Protein Is inhibited by inhibiting Megalin/Cubulin Pathway

Statins regulate Megalin Cubulin activity via Rho and RAS signalling pathways
Rosuvastatin protects Proximal Tubule against Polymyxin B Toxicity

Co-exposure of cells to polymyxin B and rosuvastatin results in

- Significantly less release of Kim 1
- Significant increase in monolayer Integrity (TEER)
- Significant effect on cell viability (MTS assay)
Rosuvastatin protects human Proximal Tubule against Polymyxin B Toxicity

Co-exposure of cells to polymyxin B and rosuvastatin results in:

- Significantly less release of Kim 1
- No significant effect on monolayer Integrity (TEER) remained ~90% control ± rosuvastatin
- No significant effect on cell viability (MTS assay) remained ~90% control ± rosuvastatin
Human and Rat Nephrotoxicity Screening: Multiple options for measuring endpoints of acute injury

<table>
<thead>
<tr>
<th>Measures of Monolayer Integrity</th>
<th>Multiple Assay Formats</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEER values</td>
<td>Human or rat PTCs</td>
</tr>
<tr>
<td>Paracellular Permeability MTX</td>
<td>Single dose</td>
</tr>
<tr>
<td></td>
<td>Multiple dosing over 0-168 hour (7 day) window</td>
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<tr>
<td></td>
<td>Dose titration</td>
</tr>
<tr>
<td></td>
<td>Biomarker sampling from cell culture medium</td>
</tr>
<tr>
<td>BioMarkers</td>
<td>Biomarker sampling from cell lysate</td>
</tr>
<tr>
<td>KIM 1</td>
<td>Cell viability staining</td>
</tr>
<tr>
<td>CLUSTERIN</td>
<td>Protein/mRNA measurement available</td>
</tr>
<tr>
<td>NGAL</td>
<td></td>
</tr>
<tr>
<td>NAG</td>
<td></td>
</tr>
<tr>
<td>IL18</td>
<td></td>
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<tr>
<td>(TF3)</td>
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<tr>
<td>αGST</td>
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